Accelerated Discovery of Cynodon *Genes and DNA Markers by cDNA Sequencing*

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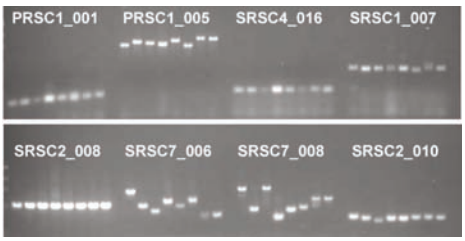
Objectives:

1. We will more than double the Internet-accessible 'gene encyclopedia' for *Cynodon* by sequencing 12,000 *Cynodon* expressed sequence tags (ESTs).
2. To nurture application of EST resources to many questions in *Cynodon* biology and improvement, we will develop a freely-available online resource of 'conserved intron scanning primers'.

Start Date: 2006
Project Duration: three years
Total Funding: $90,000

As a fringe benefit of the Human Genome Project, it has become possible to discover most of the genes that comprise the genetic blueprint of a plant or animal at manageable cost and in a short timeframe. Such 'gene encyclopedias' empower researchers to determine the role(s) of each gene in the life-cycle of an organism, identify hereditary differences among individuals, and engineer genotypes that better suit human needs. This provides an alternative to controversial biotechnology approaches using genetically modified organisms (GMOs) and may permit society to reap the potential benefits of many research goals in a publicly acceptable and environmentally safe manner.

While most genes have been identified for some crops such as maize, rice, and sorghum, *Cynodon* (bermudagrass) lags far behind. Our recently completed USGA project yielded sequences for portions of nearly 5,000 *Cynodon* genes, and a partner project (A. Guenzi) has yielded nearly 4,000 more for a total of about 9,000. While seemingly large, these numbers are tiny in comparison to 868,456 known for wheat, 472,163 for barley, and similarly high numbers for many other crops (per GenBank, as of this writing). Studies in other organisms suggest that EST sequencing is the most cost-effective gene discovery method up to at least 100,000 sequences. In other words, there is much more to learn about *Cynodon* genes from this efficient approach.



Examples of eight conserved-intron-scanning primer PCR products using the same primers for (from left in each set) rice, maize, sorghum, pearl millet, tef, bermudagrass, wheat, and barley.

We have more than doubled knowledge of the *Cynodon* transcriptome (set of genes that encode an mRNA) to about 20,000 ESTs (sequences for portions of genes). While still far smaller than the numbers available for many other crops, this will considerably improve our knowledge of the *Cynodon* gene set, identifying many additional genes for which functions can be deduced based on analogy to genes with similar sequence ('spelling') in other plants.

The resulting increased number of genes will be sufficient to begin pursuing important applications with a reasonable expectation of success, some of which we elaborate on below. Further, the proposed tools are central to establishing sufficient 'critical mass' to warrant a 'turf genomics initiative' of national scope, and will immediately leverage two NSF awards in making the results web-accessible and user-friendly.

A *Cynodon* 'gene encyclopedia' will provide the community with the complete set of tools needed to locate and isolate important and unique *Cynodon* genes, setting the stage for a new era in breeding and genetics research in *Cynodon* and other turfgrasses.

In addition to enriching knowledge of the *Cynodon* gene set, we have also provided on-line resources to foster development of large numbers of DNA markers suitable for a wide range of studies. Conserved intron scanning primers (CISP) provide large numbers of PCR-based 'pan-grass' tools suitable for linking genomics research in many crops of critical economic importance but which lack appreciable sequence information (such as *Cynodon*), to burgeoning knowledge in botanical models and better-studied crops. Because CISPs are based on PCR, they require little DNA. This makes them suitable to varying levels of technology and cost associated with practice in a wide range of scenarios ranging from high-throughput application in breeding programs to targeted study in molecular biology labs.

Because CISPs are designed within genes, this approach permits us to target variation in genes directly, rather than indirectly via a proxy DNA marker. This increases the likelihood of finding the specific mutation responsible for a trait, rather than merely a diagnostic tool.

Summary Points

- This project increased the number of *Cynodon* gene sequences to about 20,000, more than doubling our knowledge of its genes and their functions.
- Identification of corresponding rice/sorghum genes will permit scientists to deduce the probable functions of many *Cynodon* genes and also reveal features that are present in the genomic DNA that surrounds *Cynodon* genes but are absent from the ESTs, such as the 'promoters' (on/off switches) that regulate their expression.
- Large numbers of 'conserved intron scanning primers,' (PCR-based markers) are ideal for many applications in *Cynodon* research and improvement.
- While a complete sequence of the *Cynodon* genome remains far in the future, our work will build the framework for efficient progress by linking this project, the most extensive *Cynodon* sequencing effort, to the first detailed *Cynodon* genetic map.